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Recent advances in *Neospora* and neosporosis

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Abstract

Neospora caninum has emerged as a major cause of abortion in cattle in many countries. This paper reviews recent advances in the life cycle and biology of *Neospora* with the emphasis on bovine neosporosis. The role of the recently discovered oocyst stage of *N. caninum* in the epidemiology of neosporosis is discussed. Progress made in serologic diagnosis of *N. caninum* infection is discussed. There is no vaccine for preventing *Neospora*-induced abortions in cattle or to prevent oocyst shedding in dogs. Published by Elsevier Science B.V.

Keywords: *Neospora caninum*; Neosporosis; Bovine; Equine; Canine; Oocyst; Epidemiology; Economics

1. Introduction

Neosporosis has emerged as a serious disease of cattle and dogs during the last five years. Since the first recognition of the disease in 1984 (Bjerkås et al., 1984) and the description of the new genus *Neospora* and the type species *N. caninum* (Dubey et al., 1988a), over 250 publications have appeared in the literature. An earlier review in 1996 covered its history and biology in detail, and listed 193 references (Dubey and Lindsay, 1996). Therefore, in the present paper most of the references prior to 1996 are not repeated. In this review, recent advances in the life cycle and biology of *Neospora*are discussed, with the emphasis on bovine neosporosis.

2. Life cycle and general biology

Neospora caninum is the type species of the genus. Recently, another species, *N. hughesi* has been proposed for the parasite in the horse (Marsh et al., 1998); it will be discussed

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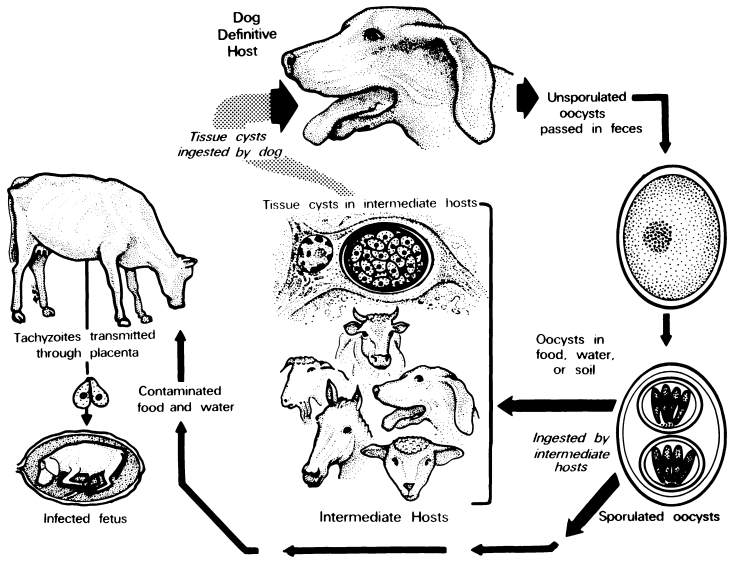

Fig. 1. Life cycle of *Neospora caninum*.

under the section on neosporosis in horses. The predicted coccidian nature of the parasite was recently confirmed (Fig. 1) when its oocyst was found in dog feces (McAllister et al., 1998a). Thus, dogs can serve both as intermediate and definitive hosts. In addition to dogs, cattle and horses discussed in this review, *N. caninum* has been found in tissues of sheep (Dubey et al., 1990), goats (Dubey et al., 1992; Barr et al., 1992), and deer (Woods et al., 1994; Dubey et al., 1996b). Antibodies to *N. caninum* were demonstrated in sera of naturally exposed water buffaloes, coyotes, red foxes, and camels suggesting that these hosts are also natural intermediate hosts for *N. caninum* (Lindsay et al., 1996a; Buxton et al., 1997c; Simpson et al., 1997; Dubey et al., 1998c; Hilali et al., 1998; Huong et al., 1998). Cats, mice, pigs, rats, gerbils, foxes, and monkeys may be induced to be experimental intermediate hosts. Tachyzoites and tissue cysts are the stages found in the intermediate hosts and both are intracellular. *Neospora caninum* tachyzoites are approximately 6×2 μm. Tissue cysts are round to oval in shape, up to 107 μm long and are found in the central nervous system including the retina (Dubey et al., 1988a; Dubey and Lindsay, 1996). The cyst wall is up to 4 μm thick and the enclosed bradyzoites are 7×2 μm. Tissue cysts have also been found in peripheral nerves of a horse (Daft et al., 1996) and once in an ocular muscle of a congenitally infected foal (Lindsay et al., 1996b).

Little is known concerning the development and tissue distribution of *N. caninum* in animals that become infected via natural routes of transmission. Tissue cysts were found in the brains of parenterally inoculated mice as early as 17 days post inoculation (McGuire et al., 1997b). Most tissue cysts were about 30 μm in diameter, although some were 107 μm (McGuire et al., 1997b) and they could be separated from mouse brain homogenate using a Percoll gradient (McGuire et al., 1997a). *Neospora caninum* has also been demonstrated in bovine fetal brain 31 days post-inoculation of the dams with tachyzoites (Dubey et al., 1992; Barr et al., 1994b). Little is known of the oral infectivity of tissue cysts and tachyzoites

for carnivores following ingestion or oral inoculation, although McAllister et al. (1998b) reported that tissue cysts derived from experimentally infected mice were not infective to cats when given orally.

Dogs fed tissue cysts may shed unsporulated oocysts (McAllister et al., 1998a; Lindsay et al., 1999). Oocysts can sporulate outside the host within 24 h (Lindsay et al., 1999). Sporulated oocysts contain two sporocysts each with four sporozoites. *Neospora caninum* oocysts are 10–11 μm in diameter and are morphologically indistinguishable from *Hammondia heydorni* found in canine feces, and *Toxoplasma gondii* and *Hammondia hammondi* in cat feces. It is worth noting that McAllister and colleagues (1998a) showed that only a few *N. caninum* oocysts were shed in dog feces and 1 of 3 dogs that shed oocysts did not seroconvert to *N. caninum*. *Neospora caninum* oocysts are not very infective to mice, including immunosuppressed (J.P. Dubey, unpublished).

At present, nothing is known regarding the frequency of shedding of oocysts, the survival of the oocysts in the environment, and whether canids other than domestic dogs are also definitive hosts for *N. caninum*.

Susceptible hosts may become infected by ingesting food and water contaminated with *N. caninum* oocysts from dog feces, however, this has so far only been shown experimentally in laboratory mice (McAllister et al., 1998b). Experimentally, animals may become infected lactogenically (Cole et al., 1995a) and calves have been infected orally by tachyzoites added to milk (Uggla et al., 1998). *Neospora caninum* has been transmitted from mother to fetus in cattle (Dubey et al., 1992; Barr et al., 1994b), sheep (Dubey and Lindsay, 1990; McAllister et al., 1996b; Buxton et al., 1997b, 1998), goats (Lindsay et al., 1995b), mice (Cole et al., 1995a; Long and Baszler, 1996; Liddell et al., 1999), dogs (Dubey and Lindsay, 1989b; Cole et al., 1995b), cats (Dubey and Lindsay, 1989a), monkeys (Barr et al., 1994a) and pigs (Jensen et al., 1998). At the present time there is no satisfactory small animal model to study the pathogenesis of *N. caninum* infection. While *N. caninum* is not very infective to outbred Swiss Webster mice, tissue cysts do occasionally develop (Dubey et al., 1988b). However, inbred BALB/c mice are more susceptible to the parasite (Lindsay et al., 1995a), with C57 BL/6 and BALB/c apparently being more susceptible than B10.D2 mice (Long et al., 1998). The availability of mice with selective genetic/immunologic defects is advantageous to study the immune mediation of *N. caninum* infection (Dubey and Lindsay, 1996; Sawada et al., 1997). In the mouse model, immunity to *N. caninum* is at least partially mediated by IL-12 and interferon gamma (Khan et al., 1997; Kasper and Khan, 1998). Thus the commercially available gamma-interferon knock-out mice can be used to isolate *N. caninum* from tissues of naturally infected animals (Dubey et al., 1998b). Although it would be desirable to study *N. caninum* immunity directly in ruminants, and this has been done to a limited extent (Harkins et al., 1998; Lundén et al., 1998; Marks et al., 1998), ruminant models are expensive and reagents are limited.

The mechanisms of primary and repeat congenital transmission of infection are unknown. Whether the repeat congenital infections which occur in dogs and cattle are due to relapse of the primary infection, or to reinfection, is unclear. Although *N. caninum* and *T. gondii* are structurally, genetically, and antigenically closely related (McAllister et al., 1996c; Beckers et al., 1997; Homan et al., 1997; Howe et al., 1997, 1998; Howe and Sibley, 1997; Sundermann et al., 1997; Tenter and Johnson, 1997; Carreno et al., 1998; Kasper and Khan, 1998; Lindsay et al., 1998) caution should be exercised in making generalizations based

on the biology of *T. gondii* because both parasites induce biologically distinct diseases. For example, *T. gondii* does not cause clinical disease in cattle and repeat congenital toxoplasmosis in animals other than rodents is rare. Also, the study by Long et al. (1998) illustrates different immunological control of *N. caninum* as compared with *T. gondii*.

3. Neosporosis in cattle

3.1. Prevalence

Neospora caninum affects both dairy (Dubey and Lindsay, 1996) and beef cattle (Hoar et al., 1996; Waldner et al., 1998). It is a major cause of abortion in dairy cattle in the U.S. (Anderson et al., 1991, 1995), New Zealand (Thornton et al., 1991), and the Netherlands (Wouda, 1998). Recently, bovine *N. caninum* infection has been reported from Argentina (Campero et al., 1998); Belgium (de Kruif et al., 1997); Canada (Duivenvoorden and Lusis, 1995; Paré et al., 1998); Denmark (Agerholm et al., 1997); Germany (Conraths et al., 1996; Schares et al., 1997,1998); Hungary (Hornok et al., 1998); Italy (Magnino et al., 1998); Japan (Yamane et al., 1997); Mexico (Morales et al., 1998); New Zealand (Reichel and Drake, 1996; Cox et al., 1998); Spain (Fondevila et al., 1998); Sweden (Stenlund et al., 1997); United Kingdom (Graham et al., 1996; Buxton et al., 1997a; Davison et al., 1997; Otter, 1997; Otter and Wilson, 1997; Otter et al., 1997; Caldow, 1998); United States (Dubey et al., 1997; Thurmond et al., 1997; Paré et al., 1997; Hattel et al., 1998); Zimbabwe (Jardine and Wells, 1995). Based on serologic surveys, up to 100% of cattle in some herds have been exposed to *N. caninum* (Dubey and Lindsay, 1996; Paré et al., 1996, 1997; Dubey et al., 1997; Gottstein et al., 1998; Reichel, 1998; Waldner et al., 1998).

3.2. Clinical signs

Clinical signs have only been reported in individual calves younger than two months of age. Abortion is the only clinical sign observed in adult cows. Cows of any age may abort from 3 months of gestation to term. Most *Neospora*-induced abortions occur at 5–6 months of gestation. Fetuses may die in utero, be resorbed, mummified, autolyzed, stillborn, born alive but diseased, or born clinically normal but chronically infected. Within herds, abortions may be clustered, sporadic or epidemic (Yaeger et al., 1994; Dubey and Lindsay, 1996; McAllister et al., 1996a; Moen et al., 1998; Wouda et al., 1998b). *Neospora caninum*-induced abortions occur year round. Cows with *N. caninum* antibodies (seropositive) are more likely to abort than seronegative cows (Thurmond et al., 1997; Moen et al., 1998; Wouda et al., 1998b).

Neospora caninum-infected calves may be born underweight, unable to rise and with neurologic signs. Hind limbs and/or forelimbs may be flexed or hyper-extended and neurologic examination may reveal ataxia, decreased patellar reflexes, and loss of conscious proprioception. Exophthalmia or an asymmetrical appearance of the eyes may also be observed.

3.3. Current methods of diagnosis

Presence of specific antibodies in serum from an aborted cow is only indicative of exposure to *N. caninum*. Several serologic tests can be used to detect *N. caninum* antibodies including ELISA, the indirect fluorescent antibody test (IFAT), and the direct agglutination test (Conrad et al., 1993a; Björkman et al., 1994; Paré et al., 1995; Baszler et al., 1996; Conraths et al., 1996; Dubey et al., 1996, 1997; Lally et al., 1996b; Björkman et al., 1997; Jenkins et al., 1997; Williams et al., 1997; Björkman and Lundén, 1998; Osawa et al., 1998; Packham et al., 1998; Romand et al., 1998; Wouda, 1998; Wouda et al., 1998a). Reagents for some of these tests are available commercially. Björkman et al. (1999) described an IgG avidity ELISA with the potential to discriminate between recent and chronic *N. caninum* infections in cattle. This assay is likely to become a valuable complement to IgG assays in epidemiologic studies of bovine *N. caninum* infections. Recently, Schares et al. (1999b) found with a newly developed ELISA serological differences between *N. caninum*-associated epidemic and endemic abortions. Within the group of animals tested seropositive by IFAT and immunoblot dams from herds with *N. caninum*-associated endemic abortions had significantly higher ELISA indices than dams from herds with *N. caninum*-associated epidemic abortions.

A definitive cut-off titer for serodiagnostic purposes has not been established for bovines because of the uncertainty of serologic diagnosis in chronically infected animals and the limited availability of sera from noninfected cattle. In serological assays, titer and absorbance values are dependant on antigen composition, secondary antibodies and other reagents. Further, cut-off values can be arbitrarily selected to provide sensitivity and specificity requested for the particular application. The age of the animal may also affect selection of a given cut-off value. For example, an IFAT titer of 1 : 640 (Conrad et al., 1993b) or 1 : 200 (Dubey et al., 1997) has been considered indicative of *N. caninum* infection in adult cattle, whereas much lower values (1 : 80) have been selected as cut-off values for samples from bovine fetuses (Barr et al., 1995). The situation may be different for adult cattle sera probably because the latter have been exposed to a very great diversity of antigens. Although *N. caninum* is closely related to *T. gondii*, *Sarcocystis* spp. and other apicomplexans, cross-reactivity has not been a major issue in animals experimentally-infected with *N. caninum* and related apicomplexans (Dubey et al., 1996a; Wouda et al., 1998a).

Examination of the fetus is necessary for a definitive diagnosis of neosporosis. Ideally, the entire fetus should be submitted but if this is not possible then samples from brain, heart, and liver should be examined for histopathological changes and body fluids or blood serum for serologic evaluation. Although *N. caninum* infection can cause lesions in several organs, fetal brain is the most consistently affected tissue. The most characteristic lesion is focal encephalitis characterized by necrosis and nonsuppurative inflammation. Hepatitis is more common in epidemic than sporadic abortions (Wouda et al., 1997b). Because most aborted fetuses are likely to be rapidly autolyzed, even semi-liquid brain tissue should be fixed in 10% buffered neutral formalin for histologic and immunohistochemical (IHC) examination. There are no pathognomonic gross lesions of neosporosis. Although a presumptive diagnosis may be made by examination of hematoxylin and eosin (H and E) stained sections, IHC is necessary because there are often only a few *N. caninum* present in autolyzed tissues and these are often not visible in H and E stained sections (Lindsay and Dubey, 1989; Dubey and

Lindsay, 1996). Only rarely are *N. caninum* organisms sufficiently numerous to be found in each histologic section (Dubey et al., 1998a). The sensitivity of even the most efficient method (IHC) to detect *N. caninum* in tissues is low.

Finding *N. caninum* antibody in fetal serum or precolostral calf serum indicates infection, but a negative result in a fetus is less useful as antibody synthesis in the fetus is dependent on the stage of gestation, level of exposure, and the time between infection and abortion (Barr et al., 1995; Wouda et al., 1997a).

Barr et al. (1995) found IFAT titers (1 : 80) in 50% of neosporosis-confirmed fetuses and in only 1 of 64 fetuses aborted due to other causes. Wouda et al. (1997a) found low IFAT titers (1 : 25) in 65% (31 of 48) of fetuses immunohistochemically positive for *N. caninum* and in 0 of the 39 fetuses aborted due to other causes. Thus, there is no reason to doubt that even a low IFAT titer of 1 : 25 is specific in fetuses. In congenitally infected calves, precolostral serum from live calves, and brain and spinal cord from dead calves are the best specimens for establishing diagnosis.

Considerable progress has been made in defining antigens of *N. caninum* by polyclonal antibodies (Barta and Dubey, 1992; Bjerkås et al., 1994; Hemphill, 1996; Hemphill and Gottstein, 1996; Hemphill et al., 1996; Marks et al., 1998; Harkins et al., 1998), by monoclonal antibodies (Table 1) (Cole et al., 1994; Baszler et al., 1996; Sundermann et al., 1997; Björkman and Hemphill et al., 1998; Howe et al., 1998; Schares et al., 1999a) and by molecular biological techniques (Table 2) (Ho et al., 1996, 1997a, b; Holmdahl and Mattsson, 1996; Kaufmann et al., 1996; Lally et al., 1996a; Müller et al., 1996; Payne and Ellis, 1996; Yamage et al., 1996; Hemphill et al., 1997a, b; Lally et al., 1997; Louie et al., 1997; Marsh et al., 1997; Asai et al., 1998; Ellis, 1998; Ellis et al., 1998; Fuchs et al., 1998; Gottstein et al., 1998; Hemphill et al., 1998; Liddell et al., 1998; Sasai et al., 1998; Sonda et al., 1998).

Three recombinant proteins of *N. caninum* have been used for the diagnosis of bovine neosporosis (Lally et al., 1996b; Jenkins et al., 1997; Louie et al., 1997).

Several monoclonal antibodies have been recently developed against *N. caninum* tachyzoites (Table 1), but their efficiency in immunohistochemical (IHC) identification of *N. caninum* has not been reported. There is an urgent need for a commercially available *N. caninum*-specific monoclonal antibody for IHC because polyclonal *N. caninum* antibodies sometimes cross react with *T. gondii* (Dubey and Lindsay, 1996). Although the monoclonal antibody reported by Cole et al. (1993, 1994) specifically detects *N. caninum* in tissue sections by IHC, it has not been produced commercially because of technical problems.

Several polymerase chain reaction (PCR) methods have been reported to detect *N. caninum* DNA (Ho et al., 1996, 1997a, b; Holmdahl and Mattsson, 1996; Kaufmann et al., 1996; Lally et al., 1996a; Müller et al., 1996; Yamage et al., 1996; Ellis, 1998; Ellis et al., 1998). These different PCR methods have not yet been evaluated critically for the diagnosis of *N. caninum*-induced abortion in cattle. Using the PCR method described by Müller et al. (1996), Gottstein et al. (1998) examined 83 bovine fetuses from Switzerland for protozoal abortion. *Neospora*-specific DNA was found in 24 (29%), and *T. gondii*-specific DNA was found in 4 (5%) fetuses. These findings are interesting and need confirmation because Ellis (1998) in Australia also found *T. gondii* DNA in 2 of 40 aborted bovine fetuses; *Neospora* DNA was found in 16 of the 40 fetuses. The findings by Gottstein et al. (1998) and Ellis

Table 1
Characterization of the molecular weights (kDa), immunofluorescence and immunoelectron-microscopical pattern of *Neospora caninum* tachyzoite antigens recognized by monoclonal antibodies (MAB)

MAB	Non-reduced antigen[a]	Reduced antigen[a]	Epitope is sodium *m*-periodate sensitive	Immuno-fluorescence[b]	Immunoelectron microscopy[b]	Reference
6G7	ND[c]	97.4, 90, 80, 70, 43, 38.5, 34, 31	ND	SURF	SURF (PRE), PV, DG, MIC, RHOP	Cole et al. (1994)
4A4-2	ND	65	Yes	SURF	ND	Baszler et al. (1996)
D9	ND	42	ND	INT (DG?)	DG	Sundermann et al. (1997)
Ncmab-4	30/32	30/32	No	SURF, INT	SURF, DG	Björkman and Hemphill (1998)
Ncmab-7	18	18	Yes	SURF, INT	SURF (PRE)	Björkman and Hemphill (1998)
Ncmab-10 and -17	41	41	No	INT (DG (?)	SURF (PRE)	Björkman and Hemphill (1998)
Ncmab-13 and -24	–[d]	61	No	INT (apical end)	–	Björkman and Hemphill (1998)
5H5 and 4H7	35	ND	ND	ND	SURF	Howe et al. (1998)
6C11	29	ND	ND	ND	SURF	Howe et al. (1998)
1.11.1	40 (38, 36)	–	Yes	SURF	–	Schares et al. (1999a)
5.2.15	38 (36, 33)	–	No	SURF	SURF	Schares et al. (1999a)
4.11.5	33 (28, 42, 22)	33 (28, 24, 22)	No	INT (DG?)	DG, PV	Schares et al. (1999a)
4.7.12	19	19	No	SURF	–	Schares et al. (1999a)

[a] Values in paretheses indicate the molecular size of additional faint reactivities.
[b] SURF: surface, INT: internal, PV: parasitophorous vacuole, SURF (PRE): surface in pre-embedding immunoelectron microscopy, DG: dense granules, MIC: micronemes, RHOP: rhoptries.
[c] ND: No data.
[d] No reactivities observed.

Table 2
Cloned *Neospora caninum* antigens[a]

Name	Size (kDa)	Other name	Genbank Accession No.	Similar to *T.gondii* (or other) proteins[b]	Tachyzoite location	Bradyzoite or tissue cyst location	Reference
NCDG1	33	Nc4.1 Nc2.1 p. 33	U82229 (full length) U72991 U36386	GRA-7 31%	DG	ND (see p. 33)	Lally et al. (1997) Lally et al. (1996b)
NCDG2	37	Nc14.1	AF029350 (full length) U36387	GRA-6 47%	DG, SURF	ND	Lally et al. (1996b) Liddell et al. (1998)
14-3-3	ND	Nc13.1	U31542	14-3-3 eukaryotic family 60%	ND	ND	Lally et al. (1996a)
Nc19.2	30	Nc14.3	NYS	BAG1/ BAG1 28%	+ve by IFAT	ND	To be published by Liddell and associates.
p. 36	36	Ncp29	AF060861 (full length)	SAG1	SURF, DG, PV network potentially	Negative	Hemphill et al. (1997b)
		NcSAG1	AJ005664	>50%			Fuchs et al. (1998); Sonda et al. (1998)[c]
p. 43	43	Ncp35 NcSRS2[d]	U93870	SAG3 23%	SURF, DGRHOP, TMP	Positive	Hemphill and Gottstein (1996) Hemphill (1996); Hemphill et al. (1997a); Fuchs et al. (1998)
p33	33	NCDG1	see NCDG1	see NCDG1	DG, PV membrane, and PV network potentially	Punctate staining of interior plus possibly cyst wall	Hemphill et al. (1998); Fuchs et al. (1998)
NcNTP NTPase[e]	67	–	AB010444	NTPase 69%	DG	ND	Asai et al. (1998)
NcSAG1 p29 Ncp29	29	p. 36	see p. 36	see p. 36	SURF	ND (see p. 36)	Howe et al. (1998)
NcSRS2 p. 35 Ncp35	35	p. 43	AF061249	SRS2 41%	SURF	ND (see p. 43)	Howe et al. (1998)
N57	34, 31, 30, 28	NCDG1	NYS	See NCDG1	See NCDG1 and p 33	See p. 33	Louie et al., 1997
N54	97, 87, 77, 67, 64 (plus minor bands of 28 to 64 and above 118)	–	U76556	–	ND	ND	Louie et al. (1997)

[a] NYS: not yet submitted, ND: no data, DG: dense granules, SURF: surface, TMP: inner and outer surface of the triple membrane pellicle.
[b] Percent identity at amino acid level.
[c] most recent paper by Sonda et al. (1998) indicates only tachyzoite surface.
[d] SRS2: surface antigen 1-related sequence 2.
[e] NTPase: nucleoside triphosphate hydrolase. *Neospora* NTPase is most similar to NTPaseI of *T. gondii*, which has been associated only with virulent strains of *T. gondi.*

(1998) suggest we should re-examine the question of whether *T. gondii* is an abortifacient in cattle as until now *T. gondii* is not considered to be so (Dubey and Beattie, 1988).

Isolation of *Neospora* in cell culture is rarely possible because most organisms in bovine fetuses die with autolysis of host cells (Conrad et al., 1993a).

3.4. Economic impact

As stated earlier, a major effect of *N. caninum* infections in cattle is abortion, and in some geographic regions up to 42.5% of abortions are attributable to neosporosis (Anderson et al., 1991, 1995). The economic impact will depend on the indirect costs, as well as on the value of fetuses lost. Indirect costs include professional help and costs associated with establishing diagnosis, re-breeding, increased lactation time, possible loss of milk yield, and replacement costs if aborted cows are culled (Thurmond and Hietala, 1996, 1997a, b). In one study in California, *Neospora*-seropositive heifers produced approximately 1 kg less milk than did their seronegative herd mates (Thurmond and Hietala, 1997b) and *Neospora*-seropositive cows were culled 6 months earlier than *Neospora*-negative cows.

There are no firm data on the economic losses due to neosporosis in the cattle industry anywhere in the world. The best available figures are that between 20 and 43% of all bovine abortions in California (Anderson et al., 1991, 1995) and in 15 to 20% in The Netherlands (Wouda et al., 1997b) are due to neosporosis. It has been estimated that economic losses in California directly related to *Neospora* abortions amount to approximately $35 million per year. According to J.P. Reynolds, a mid-term abortion probably costs the producer $600 to 1000 each [1] It is estimated that 5 to 15% of pregnancies end in abortions in Californian herds and about 33% of these are due to *Neospora*. Based on a figure of 1.2 million dairy cows in California, approximately 40,000 abortions could be due to neosporosis, providing rationale for the $35 million per year estimate. In Australia, it is estimated that neosporosis costs the dairy industry $85 million and the beef industry $25 million annually (Ellis, 1997). However, all these figures are estimates and there is an urgent need for a scientific study into the economic importance of bovine neosporosis.

3.5. Epidemiology and control

Neospora caninum is efficiently transmitted vertically in cattle, even for several generations (Björkman et al., 1996; Anderson et al., 1997; French et al., 1998; Schares et al., 1998) but horizontal transmission seems to be necessary to introduce new infections in the herd (Paré et al., 1996, 1997; Thurmond et al., 1997; Wouda et al., 1998c; French et al., 1998; Schares et al., 1998). No horizontal cow to cow transmission has been demonstrated. Until the recent discovery of the oocyst, environmental transmission of *N. caninum* to cows was unexplained. Seroepidemiologic data support the role of the dog in the life cycle of *N. caninum*(Paré et al., 1998; Sawada et al., 1998; Wouda, 1998). Although nothing is known at present regarding the frequency of shedding of *N. caninum* oocysts by canids in nature,

[1] Neosporosis: its prevalence and economic impact. Supplement to Compendium on Continuing Education for the Practicing Veterinarian. 1998; 20: 1-16. Participants in alphabetic order. Barr, B.C., Dubey, J.P., Lindsay, D.S., Reynolds, J.P., Wells, S.J.

the resistance of the oocysts, and whether dogs shed oocysts more than once, it is prudent to protect feed and water from contamination with dog feces. Dogs should not be allowed to eat aborted fetuses, fetal membranes or dead calves. There is no vaccine for preventing *Neospora*-induced abortion in cattle or to prevent oocyst shedding in dogs. Prevention of transmission of the parasite from dam to the fetus has not been demonstrated while the culling of seropositive cows as a means of reducing *N. caninum* infections in a herd has been suggested it is impractical in high prevalence herds.

4. Equine neosporosis

Neospora infections have been reported from an aborted foal (Dubey and Porterfield, 1990), a congenitally infected foal (Lindsay et al., 1996b), a 10-year-old horse (Gray et al., 1996), a 19-year-old horse with Cushing's disease (Daft et al., 1996), a 20-year-old horse with pituitary tumor (Hamir et al., 1998) and an 11-year-old Quarter Horse gelding (Marsh et al., 1996). *Neospora* organisms were isolated from the 11-year-old horse and described as a new species, *N. hughesi*, based primarily on molecular differences (Marsh et al., 1998). No differences were found between the small subunit ribosomal RNA gene from the canine, bovine and equine isolates of *Neospora*. However, in the ITS1, *N. hughesi* had seven nucleotide differences from *N. caninum*, and no structural and molecular differences were found between isolates of *Neospora* from dogs and cattle, confirming earlier findings by Holmdahl et al. (1997).

Tissue cysts of *N. hughesi* were smaller than *N. caninum* with thinner cyst walls ($\leq$1.0 μm thick), and bradyzoites were smaller than those of *N. caninum* (Marsh et al., 1998). It is, however, not clear at the present time whether *N. hughesi* is the sole species of *Neospora* that infects horses or if *N. caninum* also occurs in the horse. As mentioned, thick-walled tissue cysts characteristic of *N. caninum* were reported from a horse from California (Daft et al., 1996) and a congenitally infected foal from Wisconsin (Lindsay et al., 1996a, b).

Recently, antibodies to *N. caninum* were found in 21% of 296 horses slaughtered in the United States (Dubey et al., 1999).

5. Canine neosporosis

5.1. Prevalence and distribution

A recent serological survey in Japan reported a higher prevalence of *N. caninum* infection in dogs on dairy farms with abortions (31% of 48 dogs) compared with dogs from urban areas (7% of 198) (Sawada et al., 1998). Sera were screened at a 1 : 50 dilution in IFAT. In the most comprehensive survey reported so far, Barber et al. (1997a) tested 1554 dogs from three continents for *N. caninum* antibodies at a 1 : 50 dilution in the IFAT. The seroprevalences were 9% of 451 dogs in Australia; 20% of 414 dogs from Uruguay, South America; 0.2% of 500 dogs from the Falkland Islands; and 0 of 140 dogs from Kenya (Barber et al., 1997a). In another study, Barber et al. (1997b) reported *N. caninum* antibodies in 11% of 300 dogs from Belgium. Antibodies to *N. caninum* were found in 22% of 200 dogs from New Zealand

(Reichel et al., 1998). Cringoli et al. (1996) found IFAT antibodies in 29% of 194 dogs from Italy.

5.2. Clinical infections

A review of recent reports (Barber et al., 1996; Barber and Trees, 1996; Little, 1996; Longshore, 1996; Pumarola et al., 1996; Rasmussen and Jensen, 1996; van Ham et al., 1996; Patitucci et al., 1997; Weissenböck et al., 1997; Barber and Trees, 1998; Dubey et al., 1998b; Koudela et al., 1998; Pasquali et al., 1998; Reichel et al., 1998), indicates that most cases of clinical neosporosis in dogs were in congenitally infected young animals. An unusual presentation of neosporosis is dermatitis, so far reported in six adult dogs (Dubey et al., 1988a, 1995; Fritz et al., 1997; Perl et al., 1998; Poli et al., 1998; D.S. Lindsay, unpublished). One outstanding feature of these cases is the severe parasitism, with large numbers of tachyzoites present. Whether these cases are a result of an underlying immunodeficiency or associated with infections with particular different strains of *N. caninum* is worth investigation.

No new information is available regarding serologic diagnosis and treatment of infected dogs. Sulfonamides, pyrimethamine and clindamycin are drugs that may be attempted to treat canine neosporosis (Dubey et al., 1995; Barber and Trees, 1996). Decoquinate, an anticoccidial, killed *N. caninum* tachyzoites in cell culture (Lindsay et al., 1997). At present there is no drug effective against tissue cysts.

Determination of *N. caninum* antibodies in serum can aid diagnosis. The IFAT has been used most often to diagnose canine neosporosis with a high titer ($\geq$1 : 800) often indicative of acute infection (Barber and Trees, 1996). It should be noted, however, that high titers (1 : 12,800) were shown to persist for at least four years in two dogs (Barber and Trees, 1998) and clinical neosporosis (verified histologically and by isolation of the parasite) was diagnosed in two dogs with IFAT titers $\leq$1 : 50 (Dubey et al., 1998b). There is little information on IgM production in postnatally infected dogs. In two congenitally infected animals, IgM was not detectable at a 1 : 10 serum dilution (Dubey et al., 1998b).

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